Influence of foliar application of polyamines on growth, gas-exchange characteristics, and chlorophyll fluorescence in Bakraii citrus under saline conditions

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Abstract

The effects of polyamines (PAs) on salt stress in Bakraii (*Citrus reticulata* × *Citrus limetta*) seedlings were studied. Foliar treatments by putrescine (Put), spermidine (Spd), and spermine (Spm) (0, 0.5, and 1 mM) were applied during the salinity period (0 and 75 mM of NaCl). PA-treated seedlings showed a lower content of Na^+ and Cl^- in leaves. Application of PAs increased net photosynthetic rate in salt-stressed plants and it contributed to the enhanced growth parameters. PAs application considerably induced growth improvement in Bakraii seedlings which was found to be associated with reduced electrolyte leakage, increased relative water content, chlorophyll fluorescence parameters, activities of key antioxidant enzymes, as well as increased photosynthetic pigment concentration under saline regime. These results showed the promising use of PAs, especially of Spd and Spm, for reducing the negative effects of salinity stress and improving the growth of citrus seedlings.

Additional key words: abiotic stress; biomass; citrus; oxidative stress; photosystem II efficiency.

Introduction

Salinity is among the most significant, environmental factors responsible for substantial losses in agricultural production worldwide. It is also one of the most serious problems confronting sustainable agriculture in irrigated production systems in arid and semiarid regions (Ravindran *et al.* 2007). Nearly 20% of the world cultivated area and about half of the world irrigated lands are affected by this stress (Munns and Tester 2008). The progressive salinization of agricultural land is considered as the major environmental factor limiting plant growth and productivity of the arid and semiarid areas (Sudhir and Murthy 2004).

Citrus is grown commercially in over 50 countries and ranks as the top fruit crop in world production (García-Sánchez *et al.* 2002). Citrus plants are known to be sensitive to salts (Anjum 2007) because of the specific toxicity of Cl^- and/or Na^+ and to the osmotic effect caused by the high concentration of salts (García-Sánchez 2000).

Salinity causes various injuries in plants, such as tissue burning, yield reduction, and finally plant death (Romero-Aranda *et al.* 2001), causes leaf senescence, reduction of PSII activities (Nishihara *et al.* 2003), enhancing membrane permeability (Dhindsa *et al.* 1981), nutritional imbalance, and toxicity (Grattan and Grieve 1992). NaCl inhibits net photosynthetic rate (P_N) as a consequence of osmotic stress, which leads to a decrease in water potential and stomatal conductance (g_s), sugar accumulation, which causes feedback inhibition, and ion toxicity (*e.g.*, an excess of Na^+ and Cl^-) accompanied by a reduction of K^+ and Ca^{2+} (Koshbakht and Asgharei 2015). The reduction in P_N caused by salt stress depends on the kind and concentration of the salt, how the stress occurs, and the sensitivity of a plant (Lu *et al.* 2003). Salinity also induces generation of reactive oxygen species (ROS), such as superoxide,

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hydrogen peroxide, singlet oxygen, and hydroxyl radicals in plants (Leshem *et al.* 2007). ROS can cause toxic reactions, such as lipid peroxidation, protein degradation, and DNA mutation (McCord 2000). Salt stress alters the critical balance between the production of ROS and the quenching activity of antioxidants, resulting in oxidative stress that causes damage to plants (Hernández *et al.* 1999). Antioxidant enzymes, namely superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase, and glutathione peroxidase, and nonenzymatic antioxidants, such as ascorbic acid, glutathione, α-tocopherol, carotenoids, phenolics, and proline, which play a key role in quenching ROS, are implicated in stress tolerance (Hernández *et al.* 1999).

PAs, such as Put, Spd, and Spm, are plant growth regulators and low-molecular-mass polycations occurring in all living organisms (Kusano *et al.* 2008). Some reports have indicated the relationships between PAs and environmental stress (Galston *et al.* 1997, Bouchereau *et al.* 1999). Biosynthesis of PAs may be an integral part of

plant's response to salinity stress (Alcazar *et al.* 2010). Increasing polyamine biosynthesis might protect the plants from salinity by removing free radicals, maintaining membrane and cellular structures, keeping a cation–anion balance (Bouchereau *et al.* 1999), regulation of ion channels, and induction of ATP synthesis (Lopatin *et al.* 1994). Both mono- and dicotyledonous plants increase the accumulation of endogenous PAs under salt stress, and the pattern of PA metabolism in response to salinity seems to be dependent on plant defense systems and/or duration of exposure to salt stress. Exogenous PA application has been proposed as a convenient and effective approach for combating the salt tolerance of plants and ultimately improving crop productivity under high salinity (Chattopadhayay *et al.* 2003).

Therefore, the objectives of this work were to determine (*1*) the effect of salt stress and polyamines on the morphological and physiological changes in citrus plants, and (*2*) whether a foliar supply of PAs to Bakraii seedlings might be a strategy for increasing their salt tolerance.

Materials and methods

Plant material, growth conditions, salinity, and PAs treatments: The experiment was conducted under greenhouse conditions. Seeds of Iranian mandarin Bakraii (*Citrus reticulata* × *Citrus limetta*) were sown in air-dried soil (15 kg in each pot). The texture of the soil was loamy clay (pH 7.1, EC of 0.54 dS m^{-1}). After germination, seedlings (six-month-old plants) were transplanted into 30-cm wide plastic pots containing fine sand, then placed in a greenhouse at 25–28/17°C (day/night) temperature, maximum PAR of 800 µmol(photon) m^{-2} s^{-1}, 60/70 ± 5% relative humidity, and a 16-h photoperiod. Plants were irrigated at 2-d intervals with 0.5 L of water and fertilized with a commercial water soluble fertilizer containing macro- and micronutrients (*Floral Mixed fertilizer*, *IFO*, Italy). After the acclimation period of at least six months, (twelve months after seed germination), seedlings were sprayed at two-week interval (for the first time it was two weeks before the onset of salinity treatments) with 0 (control), 0.50, or 1.00 mM Put, Spd, and Spm, respectively, until both sides of all the leaves were completely wet. *Tween-20* at 1% (v/v) was added to PAs solutions and control treatment as a surfactant. Two weeks after the foliar application of PAs, all seedlings were exposed to salt treatments. For salt treatments, 75 mM NaCl was added to the pots at 3-d intervals using 0.5 L of irrigation water. To avoid osmotic shock, the NaCl concentration was increased gradually. After 60 d of the salt treatment, various analyses were performed.

Growth characteristics: At the end of experiment, after measuring the length of shoot (SL), plants were harvested and number of leaves per plant (LN) was counted. Then plants were washed with distilled water to remove adhering foreign particles. The shoots and roots fresh mass

(FM) were recorded, and the samples were dried separately at 80°C for 48 h and their dry masses (DM) were then recorded.

Leaf tissue mineral analysis: At the end of experiment, leaves of each plant were separated and washed with deionized water. Tissues were oven-dried at 70°C for 3 d and dried parts were milled to a powder for mineral nutrient analysis. Ground samples were ashed in porcelain crucibles at 550°C for 6 h. The white ash was treated in 2 ml of hot HCl, filtered into a 100-ml volumetric flask, and made up to 100 ml with distilled water. Concentration of Na$^+$ and K$^+$ were determined by flame photometry (*Model PFP7*, *Jenway,* UK). Ca^{2+} and Mg^{2+} concentrations were measured by using an atomic absorption spectrophotometer (*Perkin Elmper Analyst Model 200,* USA). Cl$^-$ was extracted from 500 mg DM of leaf tissue with 0.1 mol(HNO$_3$) L^{-1}, 0.1 M HNO$_3$ in 10% (v/v) glacial acetic acid, and samples were incubated overnight at room temperature and then filtered. Finally, Cl$^-$ concentration was determined by silver ion titration (Moya *et al.* 1999).

Assay of SOD and CAT: Frozen leaf samples (1 g) were homogenized in 50 mM sodium phosphate buffer (pH 7.8 for SOD and pH 7 for CAT). Then samples were centrifuged at 12,000 × *g* for 20 min at 4°C. The supernatant was used to measure the activity of the enzymes. SOD (EC 1.15.1.1) activity was measured according to the method described by Giannopolitis and Ries (1977). One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the rate of *p*-nitroblue tetrazolium chloride reduction at 560 nm (*U-2000, Hitachi Instruments,* Tokyo, Japan). SOD activity was expressed as unit min^{-1} mg^{-1}(protein). CAT (EC 1.11.1.6) activity

was determined according to Cakmak and Marschner (1992). The reaction mixture in a total volume of 2 ml contained sodium phosphate buffer (25 mM, pH 7) and H_2O_2 (10 mM). The reaction was initiated by the addition of 100 µl of enzyme extract and the activity was measured by determining the initial rate of disappearance of H_2O_2 at 240 nm using the extinction coefficient of 39.4 mM^{-1} cm^{-1} with a spectrophotometer (*U-2000, Hitachi Instruments,* Tokyo, Japan) for 30 s. One unit of CAT activity was defined as 1 µmol H_2O_2(decomposed) min^{-1} mg^{-1} (protein).

Starch analysis: Fresh leaf sample (the youngest fully-expanded leaves) was homogenized in 80% ethanol. After extraction, the concentration of starch was measured by the method of Hedge and Hofreiter (1962). The absorbance was measured at 630 nm using a spectrophotometer (*U-2000, Hitachi Instruments,* Tokyo, Japan). Glucose was used as standard solution.

Assay of protein: The protein was measured using bovine serum as standard according to the method of Bradford (1976).

Electrolyte leakage (EL) was used to assess membrane permeability. EL was measured using a conductivity meter (*CC-501; Elmetron*, Zabrze, Poland). Six leaf discs were taken from the youngest fully-expanded leaf on one randomly chosen plant per replicate sample (pot). After three washes with distilled water to remove surface contamination, six leaf discs were then placed in test-tube containing 10 ml of distilled water. These samples were incubated for 24 h on a shaker at room temperature. The electrical conductivity (EC) of the solution (EC1) was read after incubation. The same samples were then placed in an autoclave at 120°C for 20 min and the second EC reading (EC2) was taken after cooling the solution to room temperature. EL was then calculated as EC1/EC2, and expressed as a percentage (Lutts *et al.* 1995).

Relative water content (RWC) of leaves was determined following the method suggested by Barrs and Weatherley (1962). Ten leaf discs of 1 cm in diameter were cut from the youngest fully-expanded leaf, using a leaf punch. Leaf discs were weighed (FM) and washed three times with double distilled water and placed into a 10-ml conical flask. Leaf discs were immersed in 10 ml distilled water for 4 h at 4°C in dark. Turgid mass (TM) of leaf discs was then measured and samples were dried in hot air oven at 70°C until constant mass (DM) was achieved. RWC was estimated using the following equation:

$$\text{RWC (\%)} = [(\text{FM} - \text{DM})/(\text{TM} - \text{DM})] \times 100.$$

Chlorophyll (Chl) content was determined according to Lichtenthaler (1987). After gas-exchange measurements, Chl was extracted from 500 mg of leaf, which was chosen from the same leaf used for P_N measurements (avoiding major veins) using 80% aqueous acetone. Absorbance of

centrifuged extracts was measured at 645 and 663 nm using a spectrophotometer (*U-2000, Hitachi Instruments,* Tokyo, Japan).

Proline (Pro) was determined according to the method described by Bates *et al.* (1973). Seedlings (0.5 g fresh shoot material) were homogenized in 10 ml of 3% (v/v) aqueous sulphosalicylic acid and filtered through a *Whatman* No. 2 filter paper. The filtrate (2 ml) was then mixed with 2 ml of acid-ninhydrin reagent and 2 ml of glacial acetic acid in a test-tube and the mixture was placed in a water bath for 1 h at 100°C. The reaction mixture was extracted with 4 ml of toluene, and the chromophore-containing toluene fraction was aspirated, cooled to room temperature, and its absorbance measured at 520 nm using a spectrophotometer (*U-2000, Hitachi Instruments,* Tokyo, Japan). Appropriate Pro standards (*Sigma Chemical Co., St. Louis,* MO, USA) were included in order to calculate the concentration of Pro in each shoot tissue sample.

Chl fluorescence was measured in the dark- and light-adapted leaves between 9:00–11:00 h, with a portable fluorometer (*PAM-2500, Walz,* Effeltrich, Germany). After 30 min of dark adaptation, F_v/F_m was calculated as $(F_m - F_0)/F_m$, where F_m [induced by a short pulse (0.6 s) of saturating light of 3,450 µmol(photon) m^{-2} s^{-1}] and F_0 were the maximal and minimal fluorescence, respectively (Genty *et al.* 1989). After 4 min of illumination with continuous red, nonsaturating actinic light [447 µmol(photon) m^{-2} s^{-1}] and saturating pulses every 25 s, the maximum (F_m') and the steady state (F_s) fluorescence signals were measured in the light-adapted leaves. Then, the actinic light was turned off and the far-red pulse was applied to obtain the minimal fluorescence after the PSI excitation (F_0'). q_p was calculated as $(F_m' - F_s)/(F_m' - F_0')$ (van Kooten and Snel 1990). NPQ, which is proportional to the rate constant of the thermal energy dissipation, was estimated as $(F_m - F_m')/F_m'$ (Bilger *et al.* 2001).

Gas-exchange parameters [net photosynthetic rate (P_N), intercellular CO_2 concentration (C_i), and stomatal conductance (g_s)] were measured between 9:00–11:00 h at the end of the experiment using a portable photosynthetic system (*LI-6200, LI-COR Inc.,* Lincoln, NE, USA). Top fully expanded leaf was clamped to the leaf chamber and the observations were recorded when relative humidity (RH) and atmospheric CO_2 concentration (C_a) reached a stable value. PAR, air temperature, RH, and CO_2 concentration inside the sensor head were set at 1,300 ± 100 µmol (photon) m^{-2} s^{-1}, 31 ± 2°C, 60%, and 335–340 µmol m^{-1}, respectively, when measuring P_N.

Statistical analysis: All data are presented as means ± SD of five replicate seedlings. Statistical analyses were performed by one-way analysis of variance (*ANOVA*) using the *SAS* program version *9.1* (*SAS Inc.,* Cary, NC, USA). Differences between treatments were separated by the least significant differences (*LSD*) test at $P<0.05$.

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Results

Growth parameters: SL of salt-treated Bakraii seedlings decreased with an increasing salt content, but different foliar PAs improved the growth of the PAs-treated plants. The maximum increase of SL was recorded for the plants treated by Spd (0.5 mM) while the minimum SL was recorded for the seedlings at 75 mM NaCl without PAs treatments (Table 1). Spd was the most effective PAs in improving the SL in salinity treatment. TPFM and TPDM of the seedlings were affected by salinity. Increase of salinity from 0 to 75 mM significantly decreased the TPFM and TPDM. PAs treatments, especially Spm and Spd, caused a significant increase in TPFM and TPDM in comparison to Put treatment. The highest TPDM was recorded for the Spm- and Spd-treated seedlings with 75 mM NaCl. Although Spm increased the TPDM at 75 mM NaCl, there was no significant difference between the application of Spd and Spm at 0.5 mM. The lowest TPFM and TPDM were observed for the seedlings under 75 mM NaCl without PAs treatments (Table 1). The LN was significantly affected by increasing the salt stress from 0 to 75 Mm, with NaCl stress significantly decreasing the LN (Table 1). The leaves of seedlings treated with salt showed chlorosis and toxicity symptoms leading to leaf abscission. Application of PAs, especially of Spd (0.5 mM), significantly prevented the leaf abscission. The maximum LN was related to Spd (0.5 mM) under 75 mM NaCl, while the minimum was recorded for nontreated seedlings at 75 mM salt stress (Table 1).

Physiological and biochemical parameters: Leaf Na^+ and Cl^- concentrations were significantly elevated with NaCl (Table 2). Salinity increased K^+ and decreased Ca^{2+} and Mg^{2+} concentrations in leaves. The lowest Ca^{2+} and Mg^{2+} contents were found in control plants, not treated by PAs, at salt conditions. Leaf Ca^{2+}, Mg^{2+}, and K^+ were significantly elevated by the exogenous application of PAs. PAs resulted in the significant reduction of the Na^+ and Cl^- contents of the leaves. Spd significantly decreased the Na^+ and Cl^- contents in leaves under 75 mM NaCl (Table 2). Salt stress significantly increased EL, with maximum values observed in plants grown with 75 mM NaCl. The application of PAs significantly decreased EL in leaf discs, with the largest decrease in EL measured when 0.50 mM Spd was applied (Table 3). RWC and Chl were significantly affected by both salinity and PAs pre-treatment (Table 3). Increasing the concentrations of NaCl from 0 to 75 mM lowered RWC and Chl in Bakraii plants (Table 3). All concentrations of PAs increased RWC and Chl significantly compared with the control. However, 0.50 mM Spd was the most effective PA (Table 3). Increasing the concentration of salt resulted in higher leaf Pro (Table 3). The highest Pro was recorded for PA-nontreated seedlings at 75 mM NaCl, while the lowest content was related to the Spd-treated seedlings (0.5 mM) under 75 mM NaCl. In 75 mM NaCl, the amount of Pro in Bakraii leaves decreased by application of PAs and Spd showed the highest impact (Table 3). The protein and

Table 1. Growth characteristics of Iranian mandarin Bakraii seedlings treated with PAs and NaCl. Each value was mean ± SD (n = 5). Mean values followed by *the same letters* within a column are not significantly different at $p<0.05$. Analysis of variance (*ANOVA*); *** – significant ($P<0.001$); ** – significant ($P<0.01$). C – control; LN – leaf number; PAs – polyamine; Put – putrescine; S – 75 mM NaCl; SL – shoot length; Spd – spermidine; Spm – spermine; TPDM – total plant dry mass; TPFDM – total plant fresh mass; CV – coefficient of variation.

Treatment	LN	TPDM [g]	TPFM [g]	SL [cm]
C	30.00 ± 2.44[bcd]	14.91 ± 1.63[c]	49.71 ± 2.44[c]	37.41 ± 1.70[cd]
Put 0.5	30.10 ± 1.63[bcd]	15.80 ± 2.44[bc]	51.65 ± 0.82[bc]	38.97 ± 1.70[bc]
Put 1	27.70 ± 1.63[de]	15.31 ± 1.63[bc]	50.39 ± 1.63[bc]	38.06 ± 1.50[bcd]
Spd 0.5	37.62 ± 0.82[a]	18.40 ± 1.63[a]	55.81 ± 4.08[a]	41.91 ± 1.63[a]
Spd 1	28.80 ± 1.63[cd]	15.23 ± 1.61[bc]	52.90 ± 2.45[abc]	39.17 ± 1.60[bc]
Spm 0.5	32.60 ± 1.53[b]	16.81 ± 1.60[ab]	53.70 ± 2.44[ab]	39.22 ± 1.63[bc]
Spm 1	30.88 ± 2.44[bc]	15.05 ± 0.19[c]	51.20 ± 0.82[bc]	40.29 ± 1.70[ab]
S	16.00 ± 2.40[h]	5.90 ± 0.82[g]	20.08 ± 1.63[h]	26.95 ± 1.54[g]
S + Put 0.5	24.00 ± .82[fg]	10.88 ± 0.82[de]	34.31 ± 3.26[e]	34.76 ± 1.60[e]
S + Put 1	23.60 ± 2.04[fg]	8.65 ± 0.82[f]	25.15 ± 4.08[g]	29.97 ± 1.82[f]
S + Spd 0.5	25.80 ± 1.63[ef]	12.45 ± 1.63[d]	42.89 ± 2.45[d]	35.87 ± 1.70[de]
S + Spd 1	23.16 ± 2.32[fg]	10.09 ± 0.82[ef]	33.97 ± 2.44[e]	33.55 ± 1.60[e]
S + Spm 0.5	23.20 ± .80[fg]	11.54 ± 0.80[de]	40.60 ± 3.26[d]	33.83 ± 1.29[e]
S + Spm 1	22.00 ± 1.03[g]	8.52 ± 1.63[f]	29.05 ± 0.82[f]	30.98 ± 1.70[f]
ANOVA				
Treatment	***	**	**	***
CV [%]	19.4	8.8	9.7	6.8

Table 2. Leaf Cl⁻, Na⁺, K⁺, Ca²⁺, Mg²⁺ contents in Iranian mandarin Bakraii seedlings treated with PAs and NaCl. Each value was mean ± SD ($n = 5$). Mean values followed by *the same letters* within a column are not significantly different at $p<0.05$. Analysis of variance (*ANOVA*); *** – significant ($P<0.001$); ** – significant ($P<0.01$). C – control; PAs – polyamine; Put – putrescine; S – 75 mM NaCl; Spd – spermidine; Spm – spermine; CV – coefficient of variation.

Treatment	Cl⁻ [%]	Na⁺ [%]	K⁺ [%]	Ca²⁺ [%]	Mg²⁺ [%]
C	0.73 ± 0.02[f]	0.17 ± 0.02[f]	2.10 ± 0.16[e]	2.50 ± 0.41[c]	0.38 ± 0.02[abcd]
Put 0.5	0.50 ± 0.08[g]	0.13 ± 0.01[f]	2.48 ± 0.24[d]	2.67 ± 0.16[bc]	0.42 ± 0.16[abc]
Put 1	0.59 ± 0.08[fg]	0.14 ± 0.02[f]	2.47 ± 0.16[d]	2.53 ± 0.16[c]	0.38 ± 0.20[abcd]
Spd 0.5	0.50 ± 0.08[g]	0.11 ± 0.01[f]	2.80 ± 0.16[bcd]	3.08 ± 0.16[a]	0.48 ± 0.08[a]
Spd 1	0.52 ± 0.08[fg]	0.12 ± 0.02[f]	2.60 ± 0.16[cd]	2.83 ± 0.16[ab]	0.44 ± 0.07[ab]
Spm 0.5	0.54 ± 0.08[fg]	0.11 ± 0.02[f]	2.70 ± 0.4[bcd]	2.79 ± .24[abc]	0.42 ± 0.09[abc]
Spm 1	0.57 ± 0.08[fg]	0.13 ± 0.02[f]	2.50 ± 0.4[d]	2.63 ± 0.16[bc]	0.45 ± 0.04[ab]
S	4.10 ± 0.08[a]	2.10 ± 0.02[a]	2.50 ± 0.24[d]	1.50 ± 0.24[f]	0.20 ± 0.08[e]
S + Put 0.5	2.52 ± 0.08[d]	1.40 ± 0.32[bc]	2.90 ± 0.24[abc]	1.73 ± 0.16[def]	0.30 ± 0.16[cde]
S + Put 1	3.20 ± 0.16[b]	1.60 ± 0.16[b]	2.70 ± 0.16[bcd]	1.58 ± 0.24[ef]	0.29 ± 0.02[de]
S + Spd 0.5	2.00 ± 0.32[e]	0.90 ± 0.08[e]	3.20 ± 0.16[a]	1.92 ± 0.16[d]	0.34 ± 0.06[bcd]
S + Spd 1	2.90 ± 0.08[c]	1.20 ± 0.16[cd]	3.00 ± 0.24[ab]	1.63 ± 0.16[def]	0.30 ± 0.08[cde]
S + Spm 0.5	2.40 ± 0.32[d]	1.10 ± 0.08[de]	3.00 ± 0.24[ab]	1.86 ± 0.16[de]	0.33 ± 0.08[bcd]
S + Spm 1	3.20 ± 0.16[b]	1.30 ± 0.24[cd]	2.80 ± 0.16[bcd]	1.65 ± 0.16[def]	0.30 ± 0.02[cde]
ANOVA					
Treatment	***	***	**	***	**
CV [%]	14.4	9.7	9.3	11.6	23.4

Table 3. Leaf characteristics of Iranian mandarin Bakraii seedlings treated with PAs and NaCl. Each value was mean ± SD ($n = 5$). Mean values followed by *the same letters* within a column are not significantly different at $p<0.05$. Analysis of variance (*ANOVA*); *** – significant ($P<0.001$); ** – significant ($P<0.01$). C – control; Chl – total chlorophyll concentration; EL – electrolyte leakage; PAs – polyamine; Pro – proline content; Put – putrescine; S – 75 mM NaCl; RWC – relative water content; Spd – spermidine; Spm – spermine; CV – coefficient of variation.

Treatment	EL [%]	RWC [%]	Chl [mg g⁻¹(FM)]	Pro [μmol g⁻¹(FM)]
C	15.40 ± 1.63[f]	78 ± 1.43[bc]	0.73 ± 0.081[ab]	29.00 ± 4.89[f]
Put 0.5	13.40 ± 0.81[fg]	80 ± 4.08[ab]	0.74 ± 0.016[a]	21.14 ± 2.44[ghi]
Put 1	14.50 ± 1.63[fg]	78 ± 1.63[bc]	0.72± 0.015[ab]	27.79 ± 2.04[f]
Spd 0.5	12.21 ± 1.63[g]	82 ± 4.10[ab]	0.75 ± 0.041[a]	18.73 ± 1.63[i]
Spd 1	14.30 ± 1.60[fg]	80 ± 4.08[ab]	0.74 ± 0.032[a]	19.97 ± 0.81[hi]
Spm 0.5	12.40 ±0 .82[g]	84 ± 3.26[a]	0.75 ± 0.088[a]	24.48 ± 4.43[fgh]
Spm 1	14.70 ± 0.86[fg]	78 ± 1.63[bc]	0.76 ± 0.081[a]	25.98 ± 4.08[fg]
S	42.70 ± 3.26[a]	55 ± 4.08[h]	0.42 ± 0.016[f]	81.00 ± 5.71[a]
S + Put 0.5	33.37 ± 2.57[bc]	65 ± 4.1[efg]	0.56 ± 0.048[de]	45.22 ± 4.08[d]
S + Put 1	35.40 ± 4.08[b]	60 ± 4.08[gh]	0.50 ± 0.081[ef]	75.22 ± 4.10[b]
S + Spd 0.5	24.20 ± 1.63[e]	74 ± 3.26[cd]	0.65 ± 0.041[bc]	38.84 ± 1.63[e]
S + Spd 1	26.30 ± 1.53[e]	69 ± 7.34[de]	0.60 ± 0.082[cd]	40.17 ± 3.26[de]
S + Spm 0.5	29.95 ± 1.25[d]	66 ± 4.89[ef]	0.58 ± 0.065[cde]	42.24 ± 4.85[de]
S + Spm 1	32.35 ±0 .90[cd]	61 ± 0.82[fg]	0.56 ± 0.048[de]	60.63 ± 4.08[c]
ANOVA				
Treatment	***	***	**	***
CV [%]	14.7	13.8	12.3	10.6

starch content, SOD and CAT activity of Bakraii leaves were significantly influenced by the salt stress and PAs application (Table 4). The maximum amount of protein was observed after the Spd treatment (0.5 mM) under 75 mM NaCl, while the lowest protein content was recorded for PA-nontreated seedlings in 75 mM NaCl (Table 4). There was significant difference in starch content between control and salinity treatment. The minimum starch content was recorded for seedlings without PAs under the 75 mM NaCl. SOD and CAT activity increased significantly in leaves with increasing the salinity (Table 4). Application of PAs significantly increased the activity of the enzymes (Table 4). Among the PAs, Spd caused the highest activity of SOD and CAT at 75 mM NaCl. The minimum activity of SOD and CAT was related to PA-nontreated seedlings without salinity (Table 4). Among the PAs, the most pronounced difference was found for Spd-treated seedlings and the highest activity was recorded for the seedlings treated with Spd (0.5 mM) at 75 mM NaCl (Table 4).

Table 4. Protein, starch, antioxidant enzyme activities in leaves of Iranian mandarin Bakraii seedlings treated with PAs and NaCl. Each value was mean ± SD (n = 5). Mean values followed by *the same letters* within a column are not significantly different at $p<0.05$. Analysis of variance (*ANOVA*); *** – significant ($P<0.001$); ** – significant ($P<0.01$). C – control; CAT – catalase; PAs – polyamine; Put – putrescine; S – 75 mM NaCl; SOD – superoxide dismutase; Spd – spermidine; Spm – spermine; CV – coefficient of variation.

Treatment	Protein [mg g^{-1}(FM)]	Starch [mg g^{-1}(FM)]	SOD [U mg^{-1}(protein)]	CAT [μmol H$_2$O$_2$ mg^{-1} (protein) min^{-1}]
C	2.73 ± 0.24[c]	76.60 ± 3.26[b]	11.00 ± 0.41[j]	0.07 ± 0.02[h]
Put 0.5	2.80 ± 0.08[c]	87.60 ± 2.44[a]	15.12 ± 0.40[h]	0.09 ± 0.01[gh]
Put 1	2.77 ± 0.41[c]	57.80 ± 2.45[efg]	13.80 ± 0.41[i]	0.14 ± 0.02[e]
Spd 0.5	2.86 ± 0.41[bc]	85.30 ± 4.08[a]	17.9 ± 0.31[f]	0.11 ± 0.02[efg]
Spd 1	2.81 ± 0.16[bc]	58.60 ± 1.63[efg]	16.5 ± 0.42[g]	0.13 ± 0.08[ef]
Spm 0.5	2.77 ± 0.45[c]	67.50 ± 2.44[c]	16.5 ± 0.32[g]	0.11 ± 0.02[efg]
Spm 1	2.73 ± 0.16[c]	65.50 ± 4.08[cd]	15.12 ± 0.81[h]	0.10 ± 0.02[fgh]
S	2.06 ± 0.16[d]	22.70 ± 1.63[h]	19.00 ± 0.81[e]	0.30 ± 0.08[d]
S + Put 0.5	3.16 ± 0.21[b]	60.20 ± 4.08[ef]	22.45 ± 0.81[cd]	0.35 ± 0.02[bc]
S + Put 1	2.24 ± 0.17[d]	55.20 ± 4.18[g]	19.90 ± 0.72[e]	0.33 ± 0.03[cd]
S + Spd 0.5	3.74 ± 0.24[a]	66.30 ± 3.26[cd]	25.90 ± 0.81[a]	0.44 ± 0.02[a]
S + Spd 1	2.23 ± 0.16[d]	62.00 ± 1.63[de]	24.20 ± 0.61[b]	0.42 ± 0.01[a]
S + Spm 0.5	2.81 ± 0.18[bc]	58.20 ± 1.06[efg]	23.30 ± 0.81[bc]	0.37 ± 0.02[b]
S + Spm 1	2.69 ± 0.16[c]	56.30 ± 3.26[fg]	21.60 ± .076[d]	0.36 ± 0.02[bc]
ANOVA				
Treatment	***	***	**	***
CV [%]	7.4	6.2	9.5	12.6

Table 5. Gas-exchange characteristics in leaves of Iranian mandarin Bakraii seedlings treated with PAs and NaCl. Each value was mean ± SD (n = 5). Mean values followed by *the same letters* within a column are not significantly different at $p<0.05$. Analysis of variance (*ANOVA*); *** – significant ($P<0.001$); ** – significant ($P<0.01$). C – control; C_i – intercellular CO$_2$ concentration; g_s – stomatal conductance; PAs – polyamine; P_N – net photosynthetic rate; Put – putrescine; S – 75 mM NaCl; Spd – spermidine; Spm – spermine; CV – coefficient of variation.

Treatment	P_N [μmol(CO$_2$) m^{-2} s^{-1}]	g_s [mmol(H$_2$O) m^{-2} s^{-1}]	C_i [μmol mol^{-1}]
C	6.2 ± 0.16[b]	0.34 ± 0.02[ab]	152 ± 2.45[bc]
Put 0.5	6.4 ± 0.32[ab]	0.32 ± 0.01[abc]	158 ± 1.63[a]
Put 1	6.2 ± 0.16[b]	0.30 ± 0.02[cd]	153 ± 2.44[abc]
Spd 0.5	6.4 ± 0.16[ab]	0.33 ± 0.01[abc]	154 ± 3.26[abc]
Spd 1	6.2 ± 0.26[b]	0.30 ± 0.04[cd]	152 ± 4.08[bc]
Spm 0.5	6.6 ±0 .13[a]	0.35 ± 0.04[a]	150 ± 4.10[c]
Spm 1	6.6 ± 0.16[a]	0.31 ± 0.01[bcd]	156 ± 3.26[ab]
S	2.6 ± 0.48[g]	0.15 ± 0.04[h]	119 ± 0.82[f]
S + Put 0.5	4.0 ± 0.41[de]	0.22 ± 0.02[g]	130 ± 8.16[e]
S + Put 1	3.4 ± 0.16[f]	0.23 ± 0.02[fg]	137 ± 2.44[d]
S + Spd 0.5	4.8 ± 0.41[c]	0.28 ± 0.02[de]	139 ± 0.82[d]
S + Spd 1	3.7 ± 0.41[ef]	0.26 ± 0.01[ef]	138 ± 1.63[d]
S + Spm 0.5	4.2 ± 0.17[d]	0.25 ± 0.04[efg]	142 ± 8.16[d]
S + Spm 1	3.5 ± 0.82[f]	0.23 ± 0.03[fg]	140 ± 4.08[d]
ANOVA			
Treatment	***	**	**
CV [%]	9.9	8.2	9.4

Photosynthetic parameters: Salinity stress led to a significant decline in P_N, g_s, and C_i (Table 5). Application of PAs especially at 0.5 mM Spd significantly increased P_N, g_s, and C_i compared with the controls (Table 5). Plants treated with PAs maintained higher P_N, g_s, and C_i when subjected to salinity stress. The F_v/F_m was significantly affected by salt stress and PAs (Table 6). Salinity stress decreased F_v/F_m and F_v in the leaves of Bakraii seedlings (Tables 6, 7). The F_v/F_m in the dark-adapted state significantly decreased due to the salt-induced increase of F_0 and the marked decline of F_m (Table 6). Salt stress significantly decreased q_P in the light-adapted state, but markedly elevated NPQ (Table 7). These results indicate harmful effects of NaCl on metabolic processes ultimately resulting in reduced efficiency of PSII. PAs treatment significantly improved the Chl fluorescence parameters in Bakraii leaves (Tables 6, 7).

Table 6. Fluorescence parameters, F_0, F_m and F_v/F_m in Iranian mandarin Bakraii seedlings treated with PAs and NaCl. Each value was mean ± SD ($n = 5$). Mean values followed by *the same letters* within a column are not significantly different at $p<0.05$. Analysis of variance (*ANOVA*); *** – significant ($P<0.001$); ** – significant ($P<0.01$). C – control; F_m – maximal fluorescence yield of the dark-adapted state; F_O – minimal fluorescence yield of the dark-adapted state; F_v/F_m – maximum photochemical efficiency of PSII; PAs – polyamine; Put – putrescine; S – 75 mM NaCl; Spd – spermidine; Spm – spermine; CV – coefficient of variation.

Treatment	F_0	F_m	F_v/F_m
C	402 ± 16.32[f]	2,070 ± 1.63[a]	0.81 ± 0.01[a]
Put 0.5	410 ± 8.16[ef]	2,079 ± 8.16[a]	0.80 ± 0.04[a]
Put 1	418 ± 1.63[de]	1,980 ± 16.32[c]	0.79± 0.02[a]
Spd 0.5	410 ± 8.16[ef]	2,079 ± 0.82[a]	0.80 ± 0.01[a]
Spd 1	430 ± 8.16[d]	2,002 ± 1.63[b]	0.78 ± 0.02[a]
Spm 0.5	400 ± 16.32[f]	1,970 ± 24.49[c]	0.79 ± 0.01[a]
Spm 1	420 ± 16.32[de]	1,850 ± 40.82[d]	0.77 ± 0.02[ab]
S	510 ± 8.16[a]	1,039 ± 0.82[k]	0.51 ± 0.08[g]
S + Put 0.5	480 ± 8.20[bc]	1,400 ± 16.32[h]	0.65 ± 0.04[de]
S + Put 1	490 ± 7.30[b]	1,210 ± 8.16[j]	0.59 ± 0.01[f]
S + Spd 0.5	474 ± 5.40[c]	1,809 ± 7.34[e]	0.72 ± 0.02[bc]
S + Spd 1	484 ± 8.10[bc]	1,545 ± 4.08[f]	0.68 ± 0.02[cd]
S + Spm 0.5	510 ± 7.16[a]	1,455 ± 4.08[g]	0.65 ± 0.04[de]
S + Spm 1	489 ± 2.45[b]	1,273 ± 8.16[i]	0.60 ± 0.04[ef]
ANOVA			
Treatment	***	***	**
CV [%]	15.4	4.8	9.3

Table 7. F_v, q_P and NPQ in Iranian mandarin Bakraii seedlings treated with PAs and NaCl. Each value was mean ± SD ($n = 5$). Mean values followed by *the same letters* within a column are not significantly different at $p<0.05$. Analysis of variance (*ANOVA*); *** – significant ($P<0.001$); ** – significant ($P<0.01$). C – control; F_v – variable fluorescence; NPQ – nonphotochemical quenching; PAs – polyamine; Put – putrescine; q_P – photochemical quenching; S – 75 mM NaCl; Spd – spermidine; Spm – spermine; CV – coefficient of variation.

Treatment	F_v	q_P	NPQ
C	1,668 ± 8.16[a]	0.75 ± 0.04[a]	0.67 ± 0.02[ef]
Put 0.5	1,669 ± 1.63[a]	0.77 ± 0.02[a]	0.62 ± 0.02[gh]
Put 1	1,562 ± 1.63[b]	0.76 ± 0.05[a]	0.70 ± 0.04[de]
Spd 0.5	1,669 ± 1.63[a]	0.78 ± 0.02[a]	0.65 ± 0.04[fg]
Spd 1	1,572 ± 8.16[b]	0.77 ± 0.03[a]	0.60 ± 0.05[h]
Spm 0.5	1,570 ± 8.20[b]	0.77 ± 0.04[a]	0.67 ± 0.03[ef]
Spm 1	1,430 ± 24.49[c]	0.75 ± 0.03[a]	0.68 ± 0.01[ef]
S	529 ± 8.16[g]	0.50 ± 0.08[e]	0.98 ± 0.02[a]
S + Put 0.5	920 ± 16.32[e]	0.59 ± 0.01[d]	0.78 ± 0.03[c]
S + Put 1	720 ± 16.22[f]	0.57 ± 0.02[d]	0.85 ± 0.02[b]
S + Spd 0.5	1,335 ± 24.49[c]	0.68 ± 0.01[b]	0.69 ± 0.01[de]
S + Spd 1	1,061 ± 16.32[d]	0.65 ± 0.04[bc]	0.78 ± 0.01[c]
S + Spm 0.5	945 ± 16.32[e]	0.62 ± 0.02[cd]	0.72 ± 0.04[d]
S + Spm 1	784 ± 8.16[f]	0.60 ± 0.08[cd]	0.80 ± 0.01[c]
ANOVA			
Treatment	**	**	***
CV [%]	7.4	8.4	7.3

Discussion

PAs have various functions in all living cells, but the precise nature of their impact under stress conditions still remains a matter of debate. NaCl stress led to reduction of growth parameters in Bakraii seedlings. Similar results were reported by numerous investigators (García-Sánchez *et al.* 2002). Can *et al.* (2003) reported that irrigation plants with saline water reduced the photosynthetic capacity per unit of area as well as further depressed vegetative growth of the whole tree. Besides, Cooper and Gorton (1952) mentioned that the decrement in the growth of the plants grown under salinity conditions might be attributed to an increase in the osmotic potential of the soil solution causing a marked depression in the water-absorbing power of the plants. This study demonstrated that the exogenous application of PAs decreased the severity of the stress injury in Bakraii plants. Foliar-applied PAs due to their ability to act as growth regulators are able to modulate the plant metabolism and the production of metabolites involved in stress tolerance (Takahama and Oniki 1997). PAs are involved in plant defense to environmental stresses (Bouchereau *et al.* 1999). It has been suggested that the main role of PAs is to maintain a cation–anion balance during long-term salinity (Santa-Cruz *et al.* 1997). Hence, the use of exogenous PAs increased the physiological characteristics by increasing growth (Parvin *et al.* 2014). The individual PAs may play different roles during the plant response to salt stress. These differences may result from the differences in concentration of the PAs used and vary according to species and even cultivars of a species (Tonon *et al.* 2004).

The presence of NaCl in the water used for irrigation or in soil changes the nutritional balance of plants, leading to high ratios of Na^+/Ca^{+2}, Na^+/Mg^{+2}, and Na^+/K^+ that can cause the inhibition of the growth and yield in citrus crops. The main saline ions, Na^+ and Cl^-, can influence the nutrient absorption through competitive interactions or by influencing the ion selectivity of membranes (Behboudian *et al.* 1986). High concentration of Na^+ and Cl^- in leaves also causes substantial reduction in g_s and P_N in citrus (García-Sánchez *et al.* 2002). In the present study, K^+ concentration in leaves increased at 75 mM NaCl. Other researchers have reported increased amount of potassium under salt conditions (Behboudian *et al.* 1986). This might occur to balance the excess Cl^- in leaf cells to ensure the viability of the leaves, since K^+ is the most abundant and highly mobile (in both xylem and phloem) cation in plants (Marschner 1995). It seems that one of the mechanisms to be tolerant under the average salinity increased K^+ concentration in leaves of citrus. According to the results obtained in this experiment, we determined that with increasing salinity, the amount of Ca^{2+} in leaves decreased. Reduction in the amount of Ca^{2+} in the shoots under salt

stress can be due to the competitive effects of ions in vessels of the plant with calcium, which disrupt the transfer of this element (Botella *et al.* 1997). Ca^{2+} is important in cell biology (especially in membrane biology) during salt stress, *e.g.*, in preserving membrane integrity (Rengel 1992), signaling in osmoregulation (Mansfield *et al.* 1990), and influencing K^+/Na^+ selectivity (Cramer *et al.* 1987). Mg^{2+} decreased in leaves of Bakraii as salt concentration increased. Reports of Ruiz *et al.* (1997) and García-Sánchez (2002) show reduced contents of Mg^{2+} in shoots of different citrus cultivars under salt stress which is consistent with the results of this experiment. Very little is known about salinity and Mg^{2+} interactions in citrus. However, besides its role in Chl structure and as an enzyme cofactor, another important role of Mg^{2+} in plants is in photosynthesis, which is impaired and leads to enhanced degradation of Chl in Mg^{2+}-deficient source leaves, resulting in increased oxygenase activity of Rubisco (Marschner and Cakmak 1989). In Mg^{2+}-deficient leaves, formation of superoxide radicals and hydrogen peroxide is enhanced and therefore the leaves become highly photosensitive. Mg^{2+} deficiency may be one of the reasons for the chlorosis observed in NaCl-treated plants (Tozlu *et al.* 2000). Foliar application of PAs to salt-treated seedlings resulted in reduced Na^+ and Cl^- accumulation in leaves. Under PAs treatmets, plant K^+/Na^+ ratio is affected significantly. Maintaining K^+/Na^+ is an important mechanism for plants to adapt to salt tolerance. Our studies suggested that exogenous PAs could significantly alleviate the inhibition of growth in citrus seedlings subjected to NaCl stress and markedly improve the plant growth. Due to their polycationic nature, PAs, especially Spd, contribute to maintaining cellular cation–anion balance, and may act as a signaling regulator responsible for inward rectification of rectifying K^+ channels and certain Ca^{2+} permeable channels (Yamaguchi *et al.* 2007, Roy *et al.* 2005, Smith 1985). Consequently, exogenous PAs pretreatment might promote salinity tolerance by regulating the ion metabolism in Bakraii.

An increase in electrical conductivity indicates elevated leakiness of ions due to a loss of membrane integrity. This is an inherent feature of plants which are exposed to stresses such as salinity (Sharma *et al.* 2011). This study showed that PAs reduced the ion leakage (measured as electrolytes) in salt-stressed Bakraii plants indicating that PAs treatment facilitated the maintenance of membrane functions under stress conditions. Maintaining integrity of cellular membranes under salt stress is considered an integral part of salinity-tolerance mechanism (Stevens *et al.* 2006). This is probably due to the fact that PAs causes enhancement in the activities of antioxidant enzymes, which, in turn, protect plants against the generation of ROS and membrane injury, or may result in the synthesis of other substances, which have a protective effect on plants growing under salt stress (Upchurch 2008). In addition to their properties as free radical scavengers, PAs, particularly bound PAs, were also reported to

stabilize biological membrane by direct binding to membrane phospholipids under stress conditions (Todorova *et al.* 2007). Exogenous Spd prevented the leakage of electrolytes and amino acids and recovered the damage of plasma membrane in rice cultivars in response to salinity (Roy *et al.* 2005). RWC in leaves is known as an alternative measure of plant water status, reflecting the metabolic activity in tissues (González and González-Vilar 2001). RWC reduced by salinity which was in agreement with Stępień and Kłbus (2006). RWC reduction indicated a loss of turgor that resulted in limited water availability for the cell extension process (Katerji *et al.* 1997) and may result from lower water availability under stress conditions (Shalhevet 1993), and/or root systems which are not able to compensate for water lost by transpiration (Gadallah 2000). Exogenous application of PAs, especially Spm and Spd, noticeably alleviated the negative effects of salt stress and improved the growth of seedlings. This agrees with the findings that PAs induce tolerance to many abiotic stresses (Bouchereau *et al.* 1999). For example, exogenously applied PAs have been shown to be beneficial in sustaining growth of different crops under salt stress (Çavuşoğlu *et al.* 2007).

In this study, salinity stress caused a decrease in a protein content of leaves which confirms the report of Sharma and Dubey (2010), while foliar application of PAs increased the protein content. In plants, PAs, especially Spd and Spm, seem to play an important role *via* interacting with many anionic molecules, such as DNA, RNA, proteins, and membrane lipids due to their polycationic nature (Bouchereau *et al.* 1999). Moreover, free radicals, such as H_2O_2 accumulating under salt stress, physically distored the structure of proteins, leading to reduction of their content. Therefore, the impact of PAs might be due to either preventing the production of free radicals or free radical-scavenging mechanisms and subsequently protecting the proteins (Peltzer *et al.* 2002).

In general, under salt stress, starch contents decrease (Chaves *et al.* 2009), which is in accordance with the present study. Photosynthesis is inhibited under the stress resulting in a reduced pool of starch and soluble carbohydrates within the leaves (Demetriou *et al.* 2007). The implication of PAs, increasing the starch in leaves under the salt stress, might be due to the constructive role of PAs in improving and maintaining the structure and function of photosynthetic apparatus during salinity stress.

Results showed that under salinity stress, the Pro concentration of Bakraii leaves increased. In this regard, Zhu (2001) reported that plants accumulate compatible osmolytes such as Pro when they are subjected to salinity stress. Pro has multiple functions, such as osmotic pressure regulation, protection of membrane integrity, stabilization of enzymes/proteins, maintaining appropriate $NADP^+$/NADPH ratios, and scavenging free radicals (Hare and Cress 1997). Accumulation of Pro under stress conditions such as high salinity, in plants, has been correlated with stress tolerance (Misra and Gupta 2005). However, in our

study, PAs caused a decrease in the content of Pro in leaves showing other mechanisms, by which PAs reduced the impact of salinity stress. In this study, PAs application reduced salt-induced Pro accumulation which suggested that salt stress might be partially alleviated without requiring the accumulation of Pro to high concentrations. This appears to be supported by Jiménez-Bremont *et al.* (2006) who reported that exogenous PAs decreased Pro accumulation in salinity-exposed *Phaseolus vulgaris* L. The Pro degradation seems to be beneficial in the response to stress, given that the degradation of proline to glutamate generates reducing equivalents that support mitochondrial oxidative phosphorylation (Hare and Cress 1997). Furthermore, the proline dehydrogenase activity is capable of consuming O_2 (Hare and Cress 1997), and perhaps could reduce the oxidizing power of the cell and in turn possibly generate ROS (Rosales *et al.* 2007). On the other hand, PAs appears to be capable of mitigating damage associated with salinity stress by reducing oxidative stress and inducing Pro degradation, mechanisms that permit the plant to adapt under these conditions.

In this study, antioxidant enzyme activities (SOD and CAT) increased with the salinity. Salinity has been reported to increase SOD activity in pea plants (Hernández *et al.* 2000) and in citrus (Almansa *et al.* 2002). CAT is an important antioxidant enzyme, which catabolizes hydrogen peroxide (Larsen *et al.* 1988). Increasing activities of SOD and CAT with the application of PAs proved the role of these plant growth regulators in mitigating the adverse effects of salt stress in citrus. This finding confirms the report of Parida and Das (2005) in regard to the mutual relationship between higher antioxidant activity and salinity tolerance (Parida and Das 2005). Previous finding proposed that transgenic tobacco may significantly induce the expression of antioxidant enzymes by elevating the PAs content (Wi *et al.* 2006), resulting in tolerance to different abiotic stresses. It is evident that PAs exhibit their antioxidant effect by inducing the expression of genes encoding antioxidant enzymes. Therefore, PAs may function not only as scavengers of ROS, but also as activators of the expressions of genes encoding antioxidant enzymes (Parvin *et al.* 2014). H_2O_2 is one of the major and most stable ROS and its high concentration leads to oxidative stress through an increase of lipid peroxidation and modification of membrane permeability (Upchurch 2008); PAs minimize oxidative injury in plants by acting as direct free radical scavengers (Bors *et al.* 1989). Therefore, it seems that one mechanism, in which foliar application of PAs alleviates salt stress in Bakraii seedlings, is to increase SOD and CAT activities. Although previous findings suggested that Put might activate antioxidant enzymes and elevate antioxidants in Indian mustard (Verma and Mishra 2005) and Virginia pine seedlings under salinity stress (Tang and Newton 2005), our results showed a more important role of Spd and Spm compared to Put in alleviating salt stress in Bakraii seedlings. It is well documented that PAs counteract

oxidative damage in plants by acting as direct free radical scavengers or binding to antioxidant enzyme molecules to scavenge free radical (Bors *et al.* 1989).

In the present investigation, Chl, P_N, g_s, C_i, F_v, F_m, F_v/F_m, and q_p decreased with increasing salinity. A decrease in leaf Chl content has been described in citrus rootstocks irrigated with high NaCl concentration (Koshbakht and Asgharei 2015, García-Sánchez *et al.* 2002). This loss of Chl content could be associated with accumulation of Cl^- and Na^+ in the leaves (Anjum 2007). The decrease in leaf Chl concentration could be due to changes in the lipid protein ratio of pigment-protein complexes (Rao and Rao 1981), increased chlorophyllase activity, and degradation (Singh and Dubey 1995) and an inhibition if synthesis of photosynthetic pigments (García-Sánchez *et al.* 2002). Photosynthetic pigments present in the photosystems are believed to be damaged by stress factors resulting in a reduced light-absorbing efficiency of both photosystems (PSI and PSII) and hence a reduced photosynthetic capacity (Zhang *et al.* 2011). In the present study, salinity-induced reductions in P_N, g_s, and C_i were alleviated by exogenous PAs, in accordance with a similar experiment, implying that exogenous PAs alleviates the damage of photosynthetic apparatus under salinity conditions (Anjum 2009). Hence, the use of exogenous PAs increased the physiological characteristics of Bakraii seedlings by increasing both pigments and growth. The F_v/F_m is correlated with the efficiency of leaf photosynthesis. A decline in this ratio provides an indicator of photoinhibitory damage caused by the incident photon flux density when plants are subjected to a wide range of environmental stresses (Björkman and Demming 1987). The salt-induced increase of F_0 and the marked decline of F_m in Bakraii implied the inhibitory effects of salt ions on the potential quantum efficiency of PSII, although the elevated NPQ may serve to dissipate excess excitation energy (Wang *et al.* 2007). The reduction of F_v along an increase in F_0 is considered to be the characteristic of inhibition of the acceptor side of PSII (Šetlík *et al.* 1990). The reduction in F_v/F_m and q_p were correlated with an increase in NPQ suggesting that salt treatment induced dissipation of damaging excessive energy. This energy could be dissipated at antennae through heat emission although other mechanisms are also involved in energy dissipation, *e.g.*, related to a transmembrane H^+ gradient generated by ATPase activity and uncoupling of electron transport, which may lead to oxidative stress (Maxwell and Johnson 2000). Earlier studies have indicated that salt stress reduced P_N and g_s in the leaves of many crops (Syeed *et al.* 2011). A decline in photosynthetic capacity under salt stress is often associated with the generation of ROS (Noreen *et al.* 2010). All of these factors ultimately reduce overall growth of plants. Salt stress hinders P_N at multiple levels, such as pigments, stomatal functioning, gaseous exchange, structure and function of thylakoid membrane, electron transport, and enzyme activities, by hampering the oxidative stress mitigation mechanisms and cellular

metabolism of plants (Sudhir and Murthy 2004). Excessive salt concentrations might cause the closure of stomata, thereby decreasing the partial CO_2 pressure and C_i and consequently resulting in a decreased P_N (Bethke and Drew 1992). It is well documented that PAs are involved in protection against different environmental stresses in plants (Bouchereau *et al.* 1999). PAs exert positive effects on photosynthetic efficiency under stress conditions due to their acid–neutralizing and antioxidant properties, as well as their membrane- and cell wall-stabilizing activity (Mapelli *et al.* 2008). PAs with a high net positive charge can stabilize PSII proteins, such as D1 and D2, under photoinhibition conditions (Hamdani *et al.* 2011). PAs binding to membrane proteins may stabilize the protein structure during stress and consequently preserve photosynthetic activity (Hamdani *et al.* 2011). There have been several reports establishing that PAs, especially the thylakoid-bound PAs, participate in the regulation of structure and function of photosynthetic apparatus under environmental conditions, such as UV-B radiation (Lütz *et al.* 2005), low temperature (Sfakianaki *et al.* 2006), and salinity (Lütz *et al.* 2005, Demetriou *et al.* 2007).

Conclusion: In summary, this investigation revealed that salt stress had inhibitory effects on growth, gas-exchange characteristics, and Chl fluorescence of Bakraii seedlings. The results suggested the potential for PAs as a potent antioxidant in plants. PAs action might be, at least in part, explained by their ability to (*1*) increase the activities of SOD and CAT, (*2*) reduce the uptake of the toxic Na^+ and Cl^- and maintain ionic and osmotic balance, (*3*) scavenge ROS, to protect chlorophyll content, photosynthesis parameters, and improve photosystem functioning. PAs treatments could ameliorate the negative effect of salinity on the growth of Bakraii and PAs could be used as potential growth regulators to improve plant salinity tolerance. Spd applied at 0.50 mM was the most effective PA in providing Bakraii seedlings with salt tolerance.

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